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Role of the Breast Cancer Resistance Protein (ABCG2) in Drug Transport

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ABSTRACT

The 72-kDa breast cancer resistance protein (BCRP) is the second member of the subfamily G of the human ATP binding cassette (ABC) transporter superfamily and thus also designated as ABCG2. Unlike P-glycoprotein and MRP1, which are arranged in 2 repeated halves, BCRP is a half-transporter consisting of only 1 nucleotide binding domain followed by 1 membrane-spanning domain. Current experimental evidence suggests that BCRP may function as a homodimer or homotetramer. Overexpression of BCRP is associated with high levels of resistance to a variety of anticancer agents, including anthracyclines, mitoxantrone, and the camptothecins, by enhancing drug efflux. BCRP expression has been detected in a large number of hematological malignancies and solid tumors, indicating that this transporter may play an important role in clinical drug resistance of cancers. In addition to its role to confer resistance against chemotherapeutic agents, BCRP actively transports structurally diverse organic molecules, conjugated or unconjugated, such as estrone-3-sulfate, 17β-estradiol 17-(β-D-glucuronide), and methotrexate. BCRP is highly expressed in the placental syncytiotrophoblasts, in the apical membrane of the epithelium in the small intestine, in the liver canalicular membrane, and at the luminal surface of the endothelial cells of human brain microvessels. This strategic and substantial tissue localization indicates that BCRP also plays an important role in absorption, distribution, and elimination of drugs that are BCRP substrates. This review summarizes current knowledge of BCRP and its relevance to multidrug resistance and drug disposition.

Keywords: BCRP, ATP-binding cassette, ABCG2, transporter, drug transport

INTRODUCTION

Chemotherapy is a major form of treatment for cancers. Unfortunately, the majority of cancers are either resistant to chemotherapy or acquire resistance during treatment. The development of intrinsic or acquired resistance to a wide variety of anticancer drugs such as the anthracyclines (eg, doxorubicin), the *Vinca* alkaloids (eg, vincristine), the taxanes (eg, paclitaxel), and the topoisomerase inhibitors (eg, topotecan) is a major obstacle to successful cancer chemotherapy. One of the mechanisms by which human cancers develop multidrug resistance is the overexpression of efflux transport proteins on the plasma membrane of cancer cells. P-glycoprotein (P-gp)[1] and the multidrug resistance protein 1 (MRP1)[2] have been shown to confer resistance to a broad spectrum of chemotherapeutic agents. Recently, several other human ATP binding cassette (ABC) transporters with a potential role in drug resistance have been discovered. Among them, a novel protein, now known as the breast cancer resistance protein (BCRP)[3] or mitoxantrone-resistance protein (MXR)[4] or placenta-specific ABC protein (ABCP),[5] was cloned independently by 3 different laboratories. Immunoblotting analysis with BCRP-specific antibodies suggested that BCRP was a 72 kDa membrane protein predominantly localized to the plasma membrane of the drug-resistant cells overexpressing the transporter.[6,7] Transfection studies from various laboratories confirmed that enforced expression of BCRP cDNA in different cell types confers resistance to a variety of anticancer agents and reduces drug accumulation in the cell.[8-10] Such studies provided strong evidence that BCRP is a cause of drug resistance for certain types of chemotherapeutic agents including mitoxantrone and topotecan in tissue culture models. Since BCRP is prominently expressed in organs important for absorption (the small intestine), distribution (the placenta and blood-brain barrier), and elimination (the liver and small intestine) of drugs, an increasing amount of evidence is now emerging to support the conclusion that BCRP also plays an important role in drug disposition. In the following sections, current knowledge about the role of BCRP on drug resistance and drug disposition will be reviewed.

THE SUBFAMILY G OF ABC TRANSPORTER SUPERFAMILY

The ABC transporter superfamily is the largest protein superfamily identified to date.[11] ABC transporters are widely spread in all organisms from bacteria to mammals and are responsible for transport of a wide variety of compounds through cell membranes against concentration gradient with ATP hydrolysis as energy for the process of substrate translocation. ABC transporters have been implicated to play important roles in diverse physiological processes,[12] including transporting drugs (xenobiotics) or drug conjugates and excreting endogenous metabolites or physiological sub-

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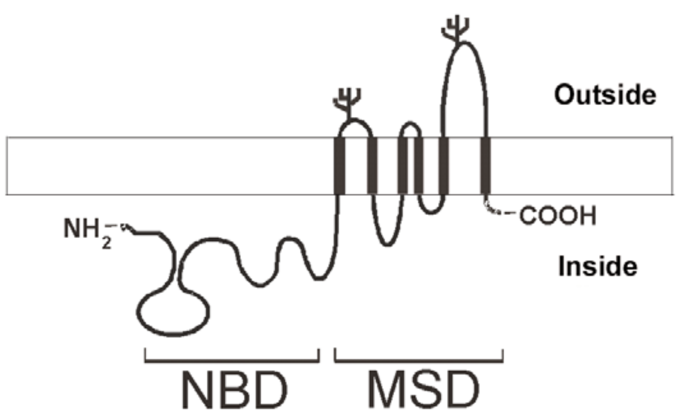

Figure 1. A membrane topology model of BCRP. BCRP contains one nucleotide binding domain (NBD) followed by one membrane-spanning domain (MSD) with 6 predicted transmembrane α-helices. Two or 3 putative N-glycosylation sites (N418, N557, or N596) are predicted to be in the extracellular loops as indicated.

strates. An increasing number of human genetic diseases are found to be associated with defects in ABC transporter genes such as CFTR in cystic fibrosis,[13] ABCR in Stargardt disease,[14] and MRP2 in Dubin-Johnson syndrome.[15] The typical structure of the majority of mammalian ABC transporters contains 2 types of structural domains: the hydrophobic membrane spanning domain (MSD) comprising several transmembrane α-helices and the hydrophilic, intracellular nucleotide binding domain (NBD). P-gp is organized in 2 tandem repeated halves of 2 domains: 1 MSD followed by 1 NBD.[1,12] The 2 repeated halves are joined by a polypeptide linker sequence. Most of mammalian ABC transporters have such a 4-domain structural organization.

BCRP belongs to a novel branch, the subfamily G, of the large ABC transporter superfamily. The founding member of ABCG subfamily is ABCG1, a human homolog of the *Drosophila* white gene.[16] BCRP is the second member of subfamily G and hence designated as ABCG2. At present, 4 human members in the subfamily G, namely, ABCG1, ABCG2, ABCG5, and ABCG8, have been identified. ABCG1 has been implicated in regulation of cellular lipid homeostasis in macrophages through facilitating efflux of cellular lipids including cholesterol and phospholipids.[16] ABCG1 may function as either homodimer or heterodimer with a yet unknown ABC transporter.[16] ABCG5 and ABCG8 have also been shown to efflux cholesterol and plant sterols.[17-19] Since both ABCG5 and ABCG8 are highly expressed in the apical membranes of small intestine and the canalicular membranes in liver, the 2 lipid transporters have been implicated in the regulation of plasma levels of dietary sterols by limiting absorption and increasing intestinal and hepatobiliary elimination of plant sterols. Recent studies indicated that ABCG5 and ABCG8 form a functional heterodimer that is essential for protein trafficking and biliary cholesterol excretion.[18] Relevant information with respect to cloning, function, and nomenclature of these proteins can be found on the Web site http://nutrigene.4t.com/humanabc.htm. Expressed sequence tag database search analyses identified 3 additional human members (ABCG4, ABCG6, and ABCG7), but their function remains elusive. Though mouse Abcg3 gene has already been cloned, human ABCG3 has not been identified thus far. Two features that distinguish the subfamily G members from other ABC transporters are aspects of their unique domain organization. The first feature pertains to the basic structure of subfamily G proteins. Comparison of ABCG protein sequences with that of P-gp and MRP1 revealed that, unlike P-gp and MRP1, which are organized in 2 repeated halves, all ABCG proteins are half transporters that are composed of a single NBD followed by one MSD (see Figure 1). There is increasing evidence to suggest that ABCG proteins may operate as either homodimers or heterodimers.[18,20] Functional expression of BCRP in Sf9-insect cells[21] and bacteria[22] support the notion that no other mammalian partners are needed for BCRP function, suggesting that BCRP may work as a homodimer or homo-oligomer. The second unique feature is the configuration of ABCG proteins in which the NBD precedes the MSD, whereas P-gp or MRP1 has an opposite domain arrangement, that is, the MSD is followed by the NBD. Such a unique domain organization is only observed in the subfamily G of human ABC transporters and implies that the transport mechanisms of ABCG proteins may be different from those of other ABC transporters.

The Breast Cancer Resistance Protein

BCRP-mediated Drug Resistance

Several cancer cell lines isolated with selection to drugs such as mitoxantrone, topotecan, and daunorubicin displayed resistance to anthracyclines, topotecan, and mitoxantrone in the absence of overexpression of P-gp or MRP1. One such cell line MCF-7/AdrVp was obtained upon long-term selection of the MCF-7 human breast cancer cell line in doxorubicin in the presence of P-gp inhibitor verapamil.[3] MCF-7/AdrVp cells do not express P-gp or MRP1 but display typical multidrug resistance phenotypes against doxorubicin with cross-resistance to daunorubicin and mitoxantrone. However, MCF-7/AdrVp cells remain sensitive to *Vinca* alkaloids, paclitaxel and cisplatin, and some of these agents are either P-gp or MRP1 substrates. In addition, MCF-7/AdrVp cells displayed ATP-dependent and reduced intracellular accumulation of daunorubicin and the fluorescent compound rhodamine 123 compared with the parental MCF-7 cells.[3] These data indicated that there exists a new efflux transporter other than P-gp or MRP1 in MCF-7/AdrVp cells. This hypothesis led to the first cloning of a new drug transporter, now known as BCRP, from the MCF-7/AdrVp cell line in 1998.[3] Sequencing analysis of BCRP cDNA confirmed that it is a novel efflux drug transporter belonging to the subfamily G of the ABC transporter superfamily.

Shortly after cloning of BCRP from MCF-7/AdrVp cells, an almost identical transporter termed MXR was discovered in a highly mitoxantrone-resistant cell line S1-M1–80, derived from the S1 human colon carcinoma cells by stepwise selection in mitoxantrone.[4] Likewise, mitoxantrone accumulation in S1-M1–80 cells was greatly reduced, whereas high levels of mitoxantrone accumulation was observed in the parental S1 cells, suggesting MXR extrudes mitoxantrone from S1-M1–80 cells. Allikmets et al[5] screened expressed sequence tag database and isolated ABCP cDNA that is essentially identical to BCRP cDNA or MXR cDNA. ABCP was so named to reflect its high level and specific expression in the human placenta. Since BCRP, MXR, and ABCP are essentially the same protein with only a few amino acid differences, we will use the original name BCRP throughout this review.

BCRP overexpression has been detected in a variety of mitoxantrone-resistant cell lines,[3,4,23] including those derived from human breast cancer (MCF-7), colon carcinoma (S1), gastric carcinoma (EPG85–257), and fibrosarcoma (EPF86–079), suggesting that BCRP overexpression is likely to be a common mechanism of mitoxantrone resistance in these cell lines. While the above-mentioned cell lines were derived by selection in mitoxantrone, BCRP-overexpression is not restricted to the cell lines selected in this agent. Several cancer cell lines made resistant to topotecan (eg, T8 and MCF-7/TPT300) also highly express BCRP with no expression of both P-gp and MRP1.[24-26] BCRP expression levels correlated closely with the levels of resistance to topotecan.[24] Topotecan accumulation in the resistant cell lines was markedly reduced presumably by enhanced drug efflux.[24-26] A topotecan-resistant cell line, T8, derived from the human ovarian cancer cell line IGROV1, displaced cross-resistance to mitoxantrone, 9-amino-camptothecin, or 7-ethyl-10-hydroxycamptothecin (SN-38), but with no or only low levels of resistance to camptothecin, doxorubicin, paclitaxel, 5′-fluorouracil (5-FU), or cisplatin.[24] MCF-7 cells selected for resistance to flavopiridol also express high levels of BCRP. The flavopiridol-resistant cell line MCF-7/FLV1000 is cross-resistant to mitoxantrone, topotecan, and SN-38 but not to 5-FU or paclitaxel.[27] Finally, transfection studies provided definitive evidence that BCRP is the cause of resistance to drugs such as mitoxantrone and topotecan in cell lines overexpressing the transporter. Drug-sensitive MCF-7 cells transfected with BCRP cDNA overexpressed BCRP protein and displayed multidrug resistance phenotype similar to that associated with drug-selected BCRP-overexpressing cell lines and reduced drug accumulation compared with the control cells transfected with empty vectors.[3] Collectively, BCRP overexpression is likely to be the cause of high-level resistance to anticancer agents including mitoxantrone, topotecan, and flavopiridol, and without resistance to paclitaxel, cisplatin, or *Vinca* alkaloids. The murine homolog of BCRP, Bcrp1, was found to confer drug resistance in a pattern very similar to that by human BCRP.[28] The porcine and rat homolog of BCRP were also cloned but their drug resistance profile has not been characterized.[29-31] The murine, rat, and porcine Bcrp1 share a high level of protein sequence identity with human BCRP (81%, 81%, and 85%, respectively).

MRP1 has been detected in intracellular vesicles and Golgi apparatus in tranfected HeLa cells,[32] resulting in altered subcellular drug distribution. This finding raises the question that, in addition to drug efflux out of the cell, intracellular sequestration and vesicular transport of drug may be an alternative mechanism of MRP1-mediated drug resistance in certain types of cells.[33] Likewise, intracellular expression of BCRP has also been detected,[32,34] although the primary localization of the transporter is at the plasma membrane in various drug-resistant cell lines.[6,7] Whether BCRP present at the intracellular membranes contributes to drug resistance remains to be determined.

Substrates of BCRP

Functional characterization in recent years has demonstrated that BCRP can transport a wide range of substrates ranging from chemotherapeutic agents to organic anion conjugates. Most of the drug-selected cell lines overexpressing BCRP display strong resistance to mitoxantrone, even if the selecting agent is not mitoxantrone.[9] Moreover, the drug-resistant cells that overexpress BCRP or the drug-sensitive cells transfected with BCRP cDNA accumulate much less mitoxantrone compared with the parental cells or drug-sensitive cells transfected with empty vectors.[28,35-37] These data suggest that mitoxantrone is a high-affinity substrate for BCRP. BCRP expression also strongly correlates with marked reduction in accumulation of and resistance to aza-anthrapyrazole (BBR3390) in BCRP-overexpressing cells.[38,39] This is as expected since BBR3390 is a mitoxantrone analog with similar anthracenedione structure.

Camptothecin derivates are the second most important class of chemotherapeutic agents that are transported by BCRP. Various BCRP-overexpressing cell lines demonstrate resistance to the camptothecin derivates including topotecan, irinotecan (CPT-11), and SN-38 (the active metabolite of irinotecan), even if some of the cell lines are not derived by selection in topotecan, and the BCRP expression levels in such cell lines are closely correlated with the levels of resistance.[25,26,40-42] Additional evidence that camptothecin derivates are BCRP substrates was obtained from BCRP cDNA transfection studies. Rabindran et al[38] observed that the MCF-7 cells transfected with BCRP cDNA displayed low but significant resistance to topotecan compared with the vector control cells and that this resistance could be reversed by the BCRP inhibitor FTC. Nakatomi et al[43] demonstrated ATP-dependent uptake of SN-38 into plasma membrane vesicles isolated from BCRP-overexpressing PC-2/SN2–5H cells, providing direct evidence that SN-38 is a substrate of BCRP. SN-38-glucuronide is also transported by BCRP but with a much lower affinity than SN-38.[43] Direct ATP-dependent transport of topotecan into BCRP-enriched plasma membrane vesicles has recently been demonstrated.[44] On the other hand, various cell lines overexpressing BCRP are not resistant to camptothecin and the camptothecin analogs including DX9851f and 9-nitrocamptothecin,[24,26,45] indicating that these compounds are either not substrates or are poor substrates of BCRP. The structural requirement for functional interaction of the camptothecin derivates with BCRP is not fully understood. Both topotecan and SN-38 contain a hydroxyl group at the 10 position of the camptothecin A ring. Rajendra et al[45] recently reported that BCRP effectively effluxes 9-aminocamptothecin but not 9-nitrocamptothecin, suggesting that the hydrophilic groups at the 9 or 10 position of the camptothecin A ring are important determinants for substrate recognition by BCRP. Various novel topoisomerase I inhibitors, homocamptothecins, with a greater potency than camptothecins, have now been discovered. These compounds differ from camptothecin in their E-ring, which is 7-membered instead of 6-membered. Both homocamptothecins and SN-38 are BCRP substrates[46]; however, HEK cells overexpressing BCRP generally displayed much less resistance to homocamptothecins than to SN-38,[46] indicating that homocamptothecins are low-affinity substrates of BCRP. These data suggest that the 7-membered E-ring present in homocamptothecins is also involved in substrate recognition by BCRP. Yoshikawa et al[47] analyzed a series of camptothecin analogs that have substitutions at positions 10 and 11 with varying polarity and found that BCRP prefers to transport the camptothecin analogs with high polarity over the analogs with low polarity. Thus, polarity seems also important for recognition of the camptothecin analogs by BCRP. All such information would be important for the design of clinically useful camptothecin analogs that are not transported by BCRP.

Flavopiridol is a cyclin-dependent kinase inhibitor currently in clinical trials. Nevertheless, cellular resistance to flavopiridol has already been observed. Robey et al[27] isolated a human breast cancer cell line highly resistant to flavopiridol by selection of the MCF-7 cells in the drug. The cell line was cross-resistant to mitoxantrone and topotecan. High levels of BCRP expression were detected in the cell line by Northern and immunoblotting analyses. Moreover, flavopiridol effectively competed with mitoxantrone for efflux in the mitoxantrone-resistant and flavopiridol-resistant cells as well as in the BCRP-transfected cells, indicating that flavopiridol is likely to be a BCRP substrate,[27] although direct transport of flavopiridol by BCRP has not been demonstrated.

Honjo et al[48] reported high levels of resistance to anthracycline in the MCF-7/AdrVp3000 and S1-M1–80 cell lines that overexpress BCRP, but not in the other 10 BCRP-overexpressing cell lines. Subsequently, sequencing BCRP cDNA revealed a Thr or Gly at position 482 in BCRP expressed in the MCF-7/AdrVp3000 and S1-M1–80 cell lines, respectively. However, all the other cell lines express BCRP with an Arg at position 482. Moreover, BCRP protein in normal tissues such as placenta has an Arg at position 482. Also, Thr or Gly at position 482 has so far not been detected in BCRP protein from clinical samples of leukemia patients.[49] Thus, BCRP with Arg at position 482 is considered as wild-type protein. Hence, anthracyclines do not appear to be transported by wild-type BCRP but are substrates of the BCRP mutants R482G and R482T. In contrast, the antifolate drug methotrexate and methotrexate polyglutamates appear to be substrates of wild-type BCRP only.[50,51] Chen et al[51] confirmed that wild-type BCRP is a high-capacity but low-affinity transporter of methotrexate with a K_m value of ~1 mM. These data suggest that amino acid at position 482 is crucial for substrate selectivity of BCRP. Although mutation at position 482 can change substrate specificity of BCRP,[48,52,53] a systematic analysis of drug-resistance phenotype revealed that compounds such as vinblastine, verapamil, and paclitaxel are not substrates of either wild-type BCRP or its mutants.[35]

Substrates of BCRP are not limited to chemotherapeutic agents. For example, BODIPY-prazosin, a fluorescent analog of the quinazoline α-blocker prazosin was shown to be transported by BCRP and it can be used to detect BCRP expression levels in cell lines.[36] BCRP is also a transporter of other fluorescent compounds. Robey et al[36] showed that rhodamine 123 and Lyso-Tracker Green are substrates of BCRP mutants R482G and R482T but not substrates of the wild-type protein. The fluorescent dye Hoechst 33342 is also an effective BCRP substrate.[54,55] Calcein-AM, an excellent substrate of P-gp, does not seem to be transported by BCRP.[35]

Recently, BCRP was shown to directly transport conjugated organic anions in transport studies using plasma membrane vesicles. BCRP is a high-affinity transporter for estrone-3-sulfate (E_1S) with K_m values of ~10 μM.[56,57] Dehydroepiandrosterone (DHEAS) is also a substrate of BCRP.[56] In addition to the sulfated conjugates, BCRP also actively transports GSH and glucuronide conjugates such as 17β-estradiol 17-(β-D-glucuronide) ($E_2 17\beta G$) and DNP-SG; however, the affinity of BCRP for sulfated conjugates appears to be greater than that of BCRP for GSH and glucuronide conjugates.[51] Hence, BCRP seems to preferentially transport sulfated conjugates of steroids and xenobiotics over GSH and glucuronide metabolites. Since E_1S and DHEAS are among the major estrogens that are synthesized and secreted by the placental syncytiotrophoblasts in the mother's body, they represent the first physiologic substrates of BCRP identified so far. BCRP can also transport unconjugated organic anions. The typical example of unconjugated organic anions that has been shown to be transported by BCRP is methotraxate. BCRP is also able to transport methotraxate conjugated with up to 3 glutamates; however, addition of even 1 more glutamate completely abrogates BCRP-mediated transport. The ability of BCRP to transport methotraxate and mono- and polyglutamate methotraxate suggests that BCRP may play a role in the maintenance of cellular folate homeostasis.[58] A free estrogen, 17β-estradiol, was reported to be a substrate for BCRP expressed in a bacteria system[22]; however, 17β-estradiol could not be transported by BCRP expressed in mammalian cells.[57] The reason for this apparent discrepancy is unknown but is likely owing to different expression systems (bacteria vs mammalian) used in the studies.

In addition to chemotherapeutic agents and organic anions, BCRP can transport a variety of chemical toxicants. These chemicals include pheophorbide a and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Pheophorbide a is a dietary chlorophyll-breakdown product that possesses phototoxicity. The Bcrp1-knockout mice displayed diet-induced photosensitivity that was accompanied with accumulation of pheophorbide a in erythrocytes.[59] This incidence of protoporphyria that occurred with Bcrp1-knockout mice is likely owing to the deficiencies in efflux of pheophorbide a by Bcrp1.[59] Indeed, direct transport of pheophorbide a by both mouse Bcrp1 and human BCRP has been demonstrated.[59,60] PhIP is a small heterocyclic amine carcinogen that plays a crucial role in the induction of various cancers such as mammary and prostate cancers.[61] Van Herwaarden et al[62] have demonstrated that PhIP is a BCRP substrate in the in vivo animal studies using Bcrp1-knockout mice. Other BCRP substrates include phosphatidylserine,[63] the HER tyrosine kinase inhibitor (TKI) CI1033,[64] the TKI STI571,[65] the flavonoid genistein,[66] the N-methyl-D-asparate (NMDA) receptor antagonist GV196771,[67] and the proton pump inhibitor pantoprazole.[68] Wang et al[69] have examined the cytotoxicity of the human immunodeficiency virus (HIV) nucleoside reverse transcriptase inhibitors zidovudine (AZT) and lamivudine (3TC) in drug-resistant MT-4/DOX_{500} (BCRP-overexpressing cells) and the parental MT-4 cells. They found that cytotoxicity of AZT and 3TC was reduced in MT-4/DOX_{500} cells compared with MT-4 cells, suggesting that AZT and 3TC may be BCRP substrates. The most recent direct transport studies have indeed confirmed that AZT and its active metabolites such as AZT 5′-monophophate are BCRP substrates.[70] Collectively, BCRP displays a broad spectrum of substrate specificity that is overlapping but distinct from that of P-gp and MRP1. For example, the anthracyclines mitoxantrone and topotecan are substrates of both P-gp and BCRP, but verapamil appears to be a substrate of only P-gp. Vincristine is a substrate of both P-gp and MRP1; however, it does not appear to be a BCRP substrate. Direct transport of therapeutic agents such as topotecan and SN-38 by BCRP has been reported,[43,44] indicating that, in contrast to MRP1, which requires GSH to cotransport certain unconjugated drugs, BCRP seems able to directly transport unmodified drugs without the need of a cofactor. Substrates of BCRP are summarized in Table 1.

Inhibitors of BCRP

A variety of BCRP inhibitors have already been identified, as summarized in Table 2. GF120918, a second-generation P-gp inhibitor, which belongs to the acridone carboxamide derivatives, is also a potent BCRP inhibitor with IC_{50} values of ~50 nM.[77,78] Various studies have shown that GF120918 can be tolerated in human and animals at concentrations sufficient to inhibit BCRP.[90-92] Thus, GF120918 is suitable for in vivo animal and clinical studies. The natural product fumitremorgin C (FTC) secreted from the fungi *Aspergillus fumigatus* was able to completely reverse mitoxantrone- and topotecan-resistance in BCRP-overexpressing cells at 1- to 5-μM concentrations.[38,72] FTC did not reverse P-gp- or MRP1-mediated drug resistance,[72] suggesting that FTC is a relatively specific inhibitor for BCRP. The molecular mechanism by which FTC inhibits BCRP is not known; however, FTC has been shown to directly inhibit BCRP-mediated transport of fluorescent substrates such as mitoxantrone and rhodamine 123 from the cell.[36,37] FTC also inhibits BCRP ATPase activity.[21] The neurotoxicity of FTC precludes its use in in vivo studies. Recently, several FTC analogs such as Ko132 and Ko134, with a much more potent inhibitory effect than FTC while displaying low in vivo toxicity, have been developed.[78,79] These compounds could be further developed as clinically useful BCRP inhibitors.

TKIs belong to a new generation of chemotherapeutic agents designed to specifically inhibit cellular signaling pathways and hence prevent cancer cell growth and metastasis. Interactions of TKIs with efflux transporters would be important for effective disposition of these drugs. Several of the TKIs have been shown to be potent inhibitors of BCRP. The HER TKI CI1033 inhibits BCRP-mediated efflux of topotecan and SN-38 at low μM concentrations, and CI1033 itself is a BCRP substrate.[64] Imatinib mesylate (STI571 or Gleevec), a phenylamino-pyrimidine, is a kinase inhibitor selective for Bcr-Ab1, activated c-Kit kinases, and platelet-derived growth factor receptor tyrosine kinase. Recently, imatinib mesylate was showed to effectively reverse topotecan resistance in BCRP-overexpressing Saos2 human osteosarcoma cells with an IC_{50} value of ~170 nM.[81] Accumulation and efflux of [^{14}C]imatinib mesylate were not

Table 1. Substrates of Breast Cancer Resistance Protein

Drug	References	Organic Molecule	References
Anthracyclines*		Fluorophores	
Daunorubicin	35,48,52	Rhodamine 123*	36,52
Doxorubicin	35,48,52	Lysotracker Green*	36,52
Epirubicin	35,71	Prazosin-BODIPY	35,52
		Hoechst 33342	55
Anthracenes			
Mitoxantrone	35,52	Conjugates	
Bisantrene	35	Estrone-3-sulfate (E_1S)	56,57
Aza-anthrapyrazole (BBR 3390)	38,39	4-MUS	56
		E3040S	56
Camptothecin Derivates		TLC-S	56
Topotecan	35	4-MUG	56
SN-38	43	E3040G	56
9-amino-camptothecin	45	$E_2 17\beta G$	51,56
Irinotecan	41,72	DNP-SG	51
Diflomotecan	73		
Polyglutamates*		Other Molecules	
Methotrexate	50,51	Phosphatidylserine	63
Methotrexate-Glu_2	51,74	Pheophorbide α	59,60
Methotrexate-Glu_3	51,74	Protoporphyrin IX	59
		PhIP	62
Nucleoside Analogs		GV196771	67
AZT	69,70	Genistein	66
AZT 5′-monophosphate	69,70		
Lamivudine (3TC)	69,70		
Other Drugs			
Prazosin	35		
Indolocarbazole	75		
Topoisomerase I inhibitors (NB-506; J-107088)	76		
Flavopiridol	27		
ErbB1 tyrosine kinase inhibitor (CI1033)	64		
Imatinib mesylate (STI571)	65		
Pantoprazole	68		

* Whether these compounds are substrates of BCRP depends on the amino acid at position 482.

different between the BCRP-expressing cells and the cells expressing a nonfunctional BCRP mutant. These data suggest that imatinib mesylate is a potent inhibitor of BCRP but itself is not a substrate for the transporter. However, the most recent study by Burger et al[65] demonstrated that imatinib mesylate is a substrate of BCRP. The reason for this apparent discrepancy is unknown but may be related to the differences in methods used by the 2 laboratories. Iressa (Gefitinib or ZD1839) is another inhibitor of HER tyrosine kinase with structure similar to CI1033. Recent studies by Özvegy-Laczka et al[80] confirmed that Iressa along with other TKIs including STI-571 and EKI-785 are potent BCRP inhibitors. Novobiocin, a coumermycin antibiotic, has also been shown to be a potent BCRP inhibitor that can sensitize BCRP-expressing cells resistant to topotecan and mitoxantrone.[44,82] In addition, Shiozawa et al[44] have shown that novobiocin inhibited ATP-dependent transport of topotecan into BCRP-enriched plasma membrane vesicles with K_i values between 26.8 and 160 nM. Using K562 human myelogenous leukemia cells transfected with BCRP cDNA, Sugimoto et al[83] demonstrated that the estrogen antagonists tamoxifen and its derivatives TAG-11 and TAG-139 had potent BCRP-reversing activity. Estrone was also found to reverse BCRP-

Table 2. Inhibitors of BCRP*

Inhibitor	IC_{50} (nM)	Reference
GF120918	50	28,77
Fumitremorgin C (FTC)	1,000	38,72
Ko132	190–270	78,79
Ko134	85–110	78,79
Iressa (Gefitinib or ZD1839)	300	80
Imatinib mesylate (STI571 or Gleevec)	170	81
EKI-785	100	80
CI1033	3700	64
Novobiocin	50–100	44,82
Estrone	ND	83
Diethylstilbestrol	ND	83
Tamoxifen, TAG-11 and TAG0139	ND	83
Reserpine	ND	84
VX-710 (Biricodar or Incel)	ND	85
Tryprostatin A	ND	86
Flavonoids (chrysin and biochanin A)	low μM ranges	66,87,88
Ritonavir	19 500	89
Saquinavir	19 500	89
Nelfinavir	12 500	89
Omeprazole	10 000–50 000	68

* Several of the inhibitors such as CI1033 are BCRP substrates.
ND indicates not determined.

mediated drug resistance.[83] Subsequently, the same group demonstrated that it was estrone-3-sulfate but not estrone that was directly transported by BCRP.[56,57] Hence, the reversing activity of estrone for BCRP is likely due to competition for efflux between estrone-3-sulfate (a metabolite of estrone in cells) and BCRP substrate drugs. Other BCRP inhibitors include reserpine (a rauwolfia alkaloid),[84] the pipecolinate derivatives VX-710 (Biricodar or Incel),[85] and tryprostatin A (an *Aspergillus fumigatus* second metabolite).[86] VX-710 increased uptake, retention, and cytotoxicity of mitoxantrone in cells overexpressing wild-type BCRP but had little effect on uptake, retention, and cytotoxicity of mitoxantrone and topotecan or SN-38 in cells expressing the BCRP mutant R482T.[85] These results suggest that VX-710 is an inhibitor of wild-type BCRP only. It should be noted that VX-710 is also an inhibitor for P-gp and MRP1.[85] Tryprostatin A would be expected as a BCRP inhibitor as it has a chemical structure similar to FTC.[86] Recently, our laboratory discovered that the HIV protease inhibitors ritonavir, saquinavir, and nelfinavir are effective inhibitors of BCRP. Ritonavir, saquinavir, and nelfinavir inhibited BCRP-mediated efflux of mitoxantrone from HEK cells stably expressing BCRP; however, none of the HIV protease inhibitors is a substrate of BCRP.[89] Zhang et al[87] recently demonstrated interactions of BCRP with a variety of food dietary flavonoids. They found that chrysin and biochanin A were the most potent BCRP inhibitors, significantly increasing mitoxantrone accumulation at concentrations of 0.5 or 1.0 μM and mitoxantrone cytotoxicity at a concentration of 2.5 μM. Recently, one of the flavoniods, genistein, has been shown to be a BCRP substrate.[66] Collectively, a large number of BCRP inhibitors with diverse chemical structures have been described. However, whether any of these compounds are clinically useful in reversing BCRP-mediated multidrug resistance has yet to be determined. We anticipate that more BCRP inhibitors will be identified along with the progress in screening substrates and/or inhibitors of the transporter. Some of the inhibitors may be used to reverse BCRP-mediated drug resistance. Application of BCRP inhibitors notably FTC and GF120918 in structure-function studies has greatly enhanced our understanding of the molecular mechanism of this important drug transporter.

Structure-Function Studies of BCRP

Earlier studies have demonstrated that amino acid at position 482 is an important determinant of substrate recognition by BCRP.[48,51,52,74,93] For example, wild-type BCRP with an Arg at position 482 does not transport daunorubicin, rhodamine 123, and Lyso-Tracker Green; in contrast, these compounds can be transported by mutants with a Thr or Gly at this position.[48] On the other hand, substances such as mitoxantrone, BODIPY-prazosin, and Hoechst 33342 are substrates of both wild-type BCRP and the 2 mutants.[48,52] A recent study by Alqawi et al[94] demonstrated that wild-type BCRP was more intensely photolabeled with IAAPh123 than the mutant with a Thr at position 482, providing direct biochemical evidence that position 482 in BCRP is critical for substrate recognition. Recent studies in our laboratory also indicate that the amino acid at position 482 has a significant effect on recognition of HIV protease inhibitors by BCRP.[89] Position 482 is predicted to be located at the COOH-proximal terminus of the third transmembrane segment (TM3) close to the cytoplasmic face (Figure 1). Thus, Arg at position 482 is likely part of the drug binding pocket located in the MSD. Since Arg is a positively charged residue, it is possible that the loss of a charged residue in the drug binding pocket can alter substrate recognition by changing salt-bridge interactions of BCRP with substrates. Thus, charged amino acids in or proximal to the MSD may be important for substrate recognition by BCRP. Recently, Miwa et al[53] generated a large number of mutants in the transmembrane segments and examined the effect of these amino acid substitutions on drug resistance conferred by BCRP. They found that amino acid substitutions of Glu at position 446, which is predicted in or proximal to the TM2 of BCRP (Figure 1), resulted in complete loss of drug resistance to SN-38 and mitoxantrone. Cells transfected with mutant BCRP cDNA with substitution of Asn residue at position 557 to Asp (N557D) exhibited comparable resistance to mitoxantrone but significantly reduced resistance to SN-38 relative to wild-type protein. Position 557 is predicted to be in or proximal to the TM5 segment. These data again provided strong evidence that the drug binding sites are likely located in the MSD and, therefore, amino acids in or proximal to the TM segments are important for substrate recognition by BCRP. Alternatively, amino acid substitutions in the TM segments may alter the membrane insertion and/or interhelical interactions and hence the substrate recognition and/or translocation pathway of the protein. The molecular basis of such observations remains to be elucidated. Position 557 is a putative N-glycosylation site (Figure 1). Whether glycosylation is important for BCRP function is not known at the present time.

A variety of naturally occurring variants of BCRP have been identified in DNA samples of ethnically diverse origins.[95-98] Notably, the alterations of BCRP protein at position 12 (V12M) and 141 (Q141K) occur frequently in Asia populations (~30%-60%) and relatively low frequencies in Caucasians and African-Americans (~5%–10%). For example, in a Japanese population studied, 39% to 50% are heterozygous and 7% are homozygous for the variant Q141K.[95,96] In a Chinese population, 60% are heterozygous for Q141K.[95] Several other variants such as I206L, N590Y, and D620N are much less frequent with allele frequencies of ~1%.[95,97] For instance, N590Y is present in ~1.5% of Caucasians.[95] I206L is found only in Hispanic populations so far.[95] D620N is detected in 1.1% of all DNA samples examined with unknown genetic origin.[97] In addition, a polymorphism in exon 4 that results in a substitution of stop codon for Gln at position 126 has also been identified.[96] Amino acid changes at position 482 that were found in some drug-selected resistant cell lines have so far not been identified in normal populations or in DNA samples from cancer patients.[49] In vitro functional characterization of the variants V12M and Q141K produced contradicting results. One study reported that Q141K was expressed at lower levels in transfected cells and therefore conferred lower drug resistance compared with the wild-type protein.[96] The variant V12M displayed expression levels and drug-resistance properties similar to the wild-type protein.[96] Another study reported that V12M and Q141K were expressed at levels comparable to the wild-type protein; however, both V12M and Q141K conferred significantly lower levels of drug resistance relative to the wild-type protein as compared with increased drug accumulation and decreased drug efflux.[98] Further analysis of the mechanism of the transport dysfunction revealed that the apical membrane localization of V12M was disrupted and that ATPase activity of Q141K was decreased.[98] A recent clinical study by Sparreboom et al[73] has shown that the Q141K polymorphism is associated with significant changes of pharmacokinetic properties of diflomotecan, a substrate of BCRP. In 5 patients heterozygous for this allele, plasma levels after intravenous drug administration were 299% of those in 15 patients with wild-type alleles. Diflomotecan levels were not significantly affected by 11 known variants in ABCB1/P-gp, ABCC2/MRP2, CYP3A4, and CYP3A5 genes. This is the first direct evidence linking an ABCG2 variant to altered drug exposure. Functional characterization of other BCRP variants has not been reported.

Recent studies suggest that BCRP may function as a homodimer[99,100] or homotetramer.[20] Kage et al[99] have constructed BCRP mutants tagged with either Myc or HA. The recombinant BCRP protein migrated as a 70 kDa protein on SDS-polyacrylamide gel under reducing condition, but as a 140 kDa complex in the absence of reducing agents. The 140 kDa complex could be immunoprecipitated with anti-Myc antibody from lysates of cells transfected with both Myc-BCRP and HA-BCRP cDNAs. The 140 kDa complex reacted with both anti-HA and anti-BCRP antibodies. After addition of reducing agents such as 2-mercaptoethanol, the 70 kDa band appeared and could be detected by immunoblotting with anti-Myc, anti-HA, and anti-BCRP antibodies. In addition, a dominant-negative mutant of BCRP with amino acid change from Leu to Pro at position 554 in the TM5 segment was found to have partially reduced the ability to confer resistance to SN-38 and mitoxantrone when cotransfected with wild-type BCRP. These studies provided direct evidence that BCRP may function as a homodimer bridged by disulfide bonds. Additional evidence that BCRP is a homodimer came from the studies by Litman et al.[100] Litman et al[100] observed a molecular mass shift of BCRP from a 72 kDa band to a 180 kDa band after treatment with cross-linking agents detected by immunoblotting using a BCRP-specific polyclonal antibody. Unexpectedly, using various conventional methods such as sucrose density gradient sedimentation and nondenaturing gel electrophoresis, Xu et al[20] recently demonstrated that BCRP may form homotetramers in the plasma membrane. Functional expression of BCRP in Sf9-insect cells,[21] bacteria,[22] and yeast[101] also provided strong evidence that BCRP may function as a homodimer or homotetramer, as no other mammalian partners are needed for BCRP function. However, Mitomo et al[102] recently showed that BCRP fully retained its ability to transport methotrexate in the presence of 2-mercatoethanol at 10 mM, a concentration that is sufficient to break down disulfide bonds between BCRP monomers. These data suggest, but do not prove, that BCRP may function as a monomer and that disulfide bond formation is not required for BCRP function. Thus, disulfide bonds do not seem to play a particularly important role in function and oligomerization of BCRP. Polgar et al[103] recently reported mutational analysis of the GXXXG motif in the first transmembrane segment TM1 of BCRP. The GXXXG motif in the MSD is presumed to be involved in dimerization of proteins such as glycoprotein A and human carbonic anhydrase. The mutants G406L, G410L, and G406L/G410L, particularly the double mutant, lost transport activity for rhodamine 123 and displayed reduced transport for mitoxantrone, pheophorbide a, and BODIPY-prazosine. Furthermore, treatment of the cells expressing the mutants with mitoxantrone resulted in increased protein expression. Thus, the GXXXG motif may be important for proper packing/folding and dimerization of the transmembrane segments in BCRP.

High levels of ATPase activity have been reported for membrane preparations isolated from insect, bacteria, and yeast cells overexpressing BCRP.[21,22,104] In addition, BCRP expressed in Sf9-insect and yeast cells can be photolabeled under hydrolytic conditions with 8-azido [α-^{32}P]ATP in the presence of vanadate and Mg^{2+} or Co^{2+},[21] indicating that BCRP is indeed able to hydrolyze ATP. Thus, BCRP functions as an energy-dependent efflux pump driven by ATP hydrolysis. For P-gp, stimulation of ATPase activity by substrates is widely believed to be directly correlated with the actual transport process.[1] However, recent studies from various laboratories[21,98] have demonstrated either inhibition or no effect on ATPase activity of wild-type BCRP by several of its substrates such as mitoxantrone, E_1S and topotecan. None of these substrates stimulated ATPase activity of wild-type BCRP. Nevertheless, substrate stimulation of ATPase activity did occur with the BCRP mutants 482T and 482G.[21] In addition, ATPase activity of BCRP expressed in bacteria was shown to be stimulated by its substrates.[22] Hence, how exactly ATP hydrolysis is coupled to the actual transport process in BCRP is poorly understood at the present time. Thus, caution should be taken in interpreting stimulation of ATPase activity by a substance or inhibitor as predictive of it being a substrate and/or inhibitor of BCRP. Taken together, although structure-function analyses of BCRP are still at an early stage, substantial progress has been made for functional characterization of BCRP with respect to substrate specificity, ATP binding and hydrolysis, and mutational analysis. Further studies are warranted for a clear understanding of the molecular mechanism by which BCRP acts to transport drugs.

BCRP Expression in Human Cancers

The expression of BCRP protein and/or mRNA has been detected in numerous types of human cancers, including hematological malignancies and solid tumors. Ross et al[105] first reported expression of BCRP mRNA measured with semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) in blast cells from 1 acute lymphoblastic leukemia (ALL) patient and 20 acute myelogenous leukemia (AML) patients. They found relatively high levels of BCRP mRNA in 7 patients with clinical resistance phenotype but not in the remaining 14 patients.[105] P-gp expression did not appear to be correlated with BCRP expression in the 7 BCRP-positive patients,[105] suggesting that BCRP-mediated transport is the likely mechanism of drug resistance. Subsequently, several other laboratories also reported BCRP expression in human leukemia patients detected with either RT-PCR or immunohistochemistry or functional assays[49,106-115]; however, whether BCRP expression is associated with relapsed/refractory leukemia remains controversial. Van der Kolk et al[109] examined 20 paired clinical samples from diagnosis and relapsed/refractory AML patients. BCRP protein levels were measured by flow cytometry with monoclonal antibodies BXP-21 and BXP-34. BCRP activity was determined by measuring mitoxantrone accumulation in the absence and presence of FTC. They found that there was no consistent upregulation of BCRP protein expression or activity with relapsed/refractory AML. In contrast, van den Heuvel-Eibrink et al[114] in a similar study showed that BCRP was the only resistance protein that was expressed at a significantly higher mRNA level with the relapsed/refractory disease relative to the diagnosis. In a study by Abbott et al,[108] the authors showed that in the blast cell samples from 40 newly diagnosed AML patients, only 7% expressed relatively high levels of BCRP mRNA and the data led the authors to conclude that high levels of BCRP mRNA expression in adult AML are relatively uncommon. Similarly, the studies by van der Pol et al[113] demonstrated that at diagnosis BCRP activity was undetectable in AML blast cells in 23 out of 26 cases, while P-gp activity was present in 36 out of 45 cases, and MRP1 activity in 26 out of 44 cases. Based upon these observations, the authors concluded that BCRP has limited function in drug resistance in AML. In childhood leukemia, no prognostic significance of BCRP expression was observed for ALL, as demonstrated in a study of 47 initial stage and 20 relapsed children with ALL.[110] Stam et al[116] compared BCRP expression in 13 infants and 13 noninfants with ALL using semiquantitative RT-PCR. They found that infants expressed 2.4-fold less BCRP mRNA than noninfants and hence concluded that BCRP does not play a significant role in drug resistance in infant ALL. However, in the case of childhood AML, BCRP expression seems to be associated with a poor response to remission induction therapy.[106] Nakanishi et al[49] analyzed 21 blast cell samples from 20 acute leukemia patients (17 AML and 3 ALL) and found that BCRP mRNA correlated proportionally with cell viability in the presence of 250 nM flavopiridol and with apoptosis induced by flavopiridol. In contrast, MDR1 mRNA level did not correlate with either flavopiridol toxicity or induction of apoptosis by flavopiridol. This led them to conclude that unlike P-gp, BCRP may play a role in leukemia resistance to flavopiridol.[49] Studies by Plasschaert et al[111] also suggest that BCRP expression is high in ALL and, more specifically, BCRP is expressed at a higher level and is functionally more active in B-lineage than in T-lineage ALL. Collectively, these studies suggest that BCRP may be involved in clinical drug resistance of some subgroups of leukemia patients. Further studies are needed to provide a more comprehensive picture of the importance of BCRP in human leukemia. Discrepancies remain among the data published by different laboratories or by the same laboratory using different methods.[117] This is likely owing to the differences in the sensitivity and accuracy of the methods (eg, RT-PCR, real-time PCR, immunohistochemistry with different antibodies, and flow-cytometry efflux assay) used to quantify BCRP expression and activity in clinical samples. Variability of patient samples may also contribute to the discrepancies.

BCRP expression has also been detected in a variety of solid tumors. Scheffer et al[118] analyzed BCRP expression in 34 untreated solid tumor samples and 7 treated solid tumors by immunohistochemistry using monoclonal antibody BXP-34 and found no BCRP expression except for one case of small intestine adenocarcinoma. Subsequently, Diestra et al[119] analyzed BCRP expression in 150 untreated human solid tumors comprising 21 tumor types using a different monoclonal antibody BXP-21 in formalin-fixed paraffin-embedded specimens. Moderate or strong expression of BCRP was seen in ~60% of the tumor samples examined. BCRP expression was observed in all tumor types with apparently higher frequency in gastric carcinoma, hepatocellular carcinoma, endometrial carcinoma, colon cancer, small cell lung cancer, and melanoma.[119] The results suggest that BCRP may represent a mechanism of drug resistance in certain types of solid tumors. To ascertain the clinical relevance of BCRP in drug resistance of solid tumors, studies for specific tumor types are needed. Kawabata et al[120] confirmed BCRP expression in 23 untreated non-small cell lung cancer (NSCLC) samples and observed that 22% of the samples expressed higher levels of BCRP mRNA than that in the NCI-H441 human lung cancer cell line with BCRP expression that confers high levels of resistance to topotecan in vitro. Most recently, Yoh et al[121] demonstrated a strong correlation of BCRP expression in tumor samples from 72 untreated stage IIIB or IV NSCLC patients with the patients' response rate to platinum-based chemotherapy. They found that expression of P-gp, MRP1, MRP2, and MRP3 were not significantly associated with response to chemotherapy or survival; however, the response rate to chemotherapy of patients with BCRP-negative tumors was 44%, whereas only 24% of patients with BCRP-positive tumors responded. Moreover, BCRP-positive patients had shorter progress-free survival and overall survival time than BCRP-negative patients.[121] These data suggest that BCRP but not P-gp or MRP1 may be a molecular target for overcoming drug resistance in advanced NSCLC patients. Candeil et al[122] analyzed BCRP expression in tumor samples from 42 patients with colon cancer and found that the BCRP mRNA levels in hepatic metastases after an irinotecan-based chemotherapy were higher than those in irinotecan-naïve metastases. The data suggest that BCRP is likely to be involved in the development of irinotecan resistance in patients with colon cancers. Though BCRP was originally cloned from a human breast cancer cell line, there is no strong evidence to suggest that BCRP is highly expressed in breast carcinoma and plays a role in drug resistance in human breast cancers.[123-125] Expression of BCRP in human cancers and its prognostic significance have been extensively reviewed recently.[9,107]

BCRP Expression and Function in Stem Cells

Zhou et al[84,126] demonstrated, for the first time, that in murine bone marrow, the "side-population" (SP) cells that are enriched for stem cells expressed the highest level of *Bcrp1* mRNA (the murine homolog of human BCRP) over other populations. The number of SP cells in either the bone marrow or liver is not changed in Mdr1a/1b$^{-/-}$ mice. However, loss of *Bcrp1* gene expression, but not *Mdr1a/1b*, led to a significant reduction in the number of SP cells in the bone marrow and in skeletal muscle.[84,126] These findings demonstrate that *Bcrp1* gene, but not *Mdr1a/1b* genes, is necessary for the SP phenotype of hematopoietic stem cells. Subsequently, functional studies demonstrated that human BCRP is highly expressed and the efflux transporter for Hoechst 33342 in the hematopoietic stem cells within the SP population region.[54,55] BCRP expression has also been observed in stem cells from a variety of other tissues such as the interstitial spaces of mammalian skeletal muscle,[127] human pancreas islets,[128] the human liver,[129] and the developing and adult heart.[130] Thus, BCRP is a molecular determinant of the SP phenotype and can be used as a marker for selection of stem cells. The precise physiological function of BCRP in stem cells remains unknown; however, the recent studies using Bcrp1$^{-/-}$ mice suggested that Bcrp1 expression in stem cells is to provide protection from cytotoxic substrates.[84,126] Indeed, Kirshnamurthy et al[131] have demonstrated that BCRP protects hematopoietic stem cells under hypoxic conditions by preventing the accumulation of heme that causes mitochondrial death, and that BCRP expression is upregulated in stem cells under hypoxic conditions via a HIF-1 signaling pathway.[131] The ability of BCRP to protect stem cells and as a selectable marker may prove useful for BCRP in gene therapy applications.[132] BCRP expression in stem cells is the topic of several recent review papers.[9,133,134]

Tissue Distribution of BCRP and Its Role in Drug Disposition

Several immunohistochemical studies using monoclonal and polyclonal antibodies have confirmed that BCRP is mainly localized to the plasma membrane of mammalian cells.[6,7,118]

This is consistent with the ability of BCRP to confer drug resistance in cancer cells by reducing drug accumulation. However, a recent study by Rajagopal and Simon[32] indicated that BCRP in transfected HeLa cells can be localized to lysosomal membranes and doxorubicin-positive intracellular vesicles. This raises the possibility that BCRP may be expressed in the cellular compartments of cancer cells where it actively sequesters drugs away from their cellular targets. Indeed, intracellular BCRP expression has recently been reported in poorly differentiated human gallbladder carcinomas.[34]

Expression of BCRP in normal tissues has been investigated. Doyle et al[3] analyzed expression pattern of BCRP mRNA by Northern blotting in a variety of normal human tissues. The highest expression of BCRP mRNA was found in the human placental tissue followed by prostate, small intestine, brain, colon, liver, and ovary. There was no detectable BCRP mRNA in tissues such as lung, skeletal muscle, kidney, pancreas, and spleen.[3] Subsequently, Maliepaard et al[7] examined cellular localization and distribution of BCRP protein expression in normal human tissues. Two different monoclonal antibodies, BXP-21 and BXP-34, were used, and both antibodies showed specific plasma membrane expression of BCRP in BCRP-overexpressing tumor cells and transfected cells. With immunohistochemical detection using BXP-21 and BXP-34, the highest expression of BCRP was observed in the placental syncytiotrophoblasts.[7] In addition, BCRP is prominently expressed in the apical membrane of the epithelium in the small intestine and colon, in the liver canalicular membrane, and in the veins and capillaries of blood vessels. BCRP expression in other tissues is relatively low. In general, the pattern of BCRP expression determined by immunohistochemistry matches well with that obtained in the Northern blot studies. Taipalensuu et al[135] showed that BCRP mRNA levels in jejunum biopsies from healthy volunteers were even greater than that of P-gp. There is a species-difference of tissue distribution for BCRP. For instance, there is little expression of BCRP in human kidney; however, murine Bcrp1 is abundantly expression in mouse kidney.[126]

Several studies have also demonstrated high level expression of BCRP in the brain. BCRP was detected by immunoblotting with BXP-21 in brain homogenates.[136] Further confocal microscopic analysis demonstrated the high level expression of BCRP at the luminal surface of the microvessel endothelium of human brain.[136] This localization closely resembles that of P-gp at the blood-brain barrier. In another study, Zhang et al[137] confirmed high BCRP expression in cultured human cerebromicrovascular endothelial cells and the human brain microvessels detected at both mRNA and protein levels. Real-time PCR showed that expression level of BCRP appeared to be higher than that of P-gp and MRP1 in both nonmalignant human brain and glioblastoma tumors. Moreover, BCRP seems to be upregulated in glioblastoma vessels relative to the normal brain.[137] Doyle and Ross[9] analyzed BCRP mRNA levels in a commercially available dot blot that contained RNA from 50 human tissues and found that certain areas of the brain, particularly the midbrain, including putamen, substantia nigra, pituitary gland, and thalamus, had high levels of BCRP mRNA. These data suggest that BCRP may be a protective efflux pump at the blood-brain barrier and other areas of the brain.

The strategic and substantial localization of BCRP in the placenta, in the small intestine, and in the liver suggests that BCRP functions as a protective efflux pump in the placenta and has the potential to limit oral absorption and increase biliary elimination of xenobiotics that are BCRP substrates. Indeed, Jonker et al[92] have shown that treatment with the BCRP inhibitor GF120918 (also a P-gp inhibitor) decreases plasma clearance and hepatobiliary excretion of topotecan and increases absorption of this anticancer drug from the small intestine in P-gp knockout mice. In pregnant GF120918-treated P-gp-deficient mice, the relative fetal concentration of topotecan was 2-fold higher than that in pregnant vehicle-treated mice. These data indicate that the Bcrp1 mediates apically directed drug transport, reduces drug bioavailability, and protects fetuses against therapeutic agents. A recent clinical study by Kruijtzer et al[91] has also demonstrated that coadministration of GF120918 significantly increases oral bioavailability of topotecan in cancer patients from 40% to 97%. Since topotecan is not extensively metabolized and is a weaker substrate for P-gp, the increase in bioavailability of topotecan, therefore, is attributable to the inhibition of BCRP by GF120918, which results in increased intestinal absorption and decreased biliary excretion of this antineoplastic agent. Thus, coadministration of a BCRP substrate drug with its inhibitor may result in clinically significant drug-drug interactions. Pantoprazole is a BCRP substrate that is able to compete for BCRP-mediated drug transport.[68] Coadministration of methotrexate with pantoprazole resulted in a 70% increase in the plasma levels of the metabolite 7-hydroxy-methotrexate in a cancer patient.[138] Thus, Breedveld et al[68] performed animal studies using Bcrp1-knockout mice to investigate the mechanism of pharmacokinetic interaction between methotrexate and pantoprazole. They found that the AUC of IV methotexate in wild-type mice was increased ~2-fold by coadministration of IV pantoprazole, while the AUC of IV methotexate in Bcrp1-knockout mice was not affected by pantoprazole. Moreover, coadministration of IV pantoprazole reduced the clearance of IV methotexate ~2-fold in wild-type mice to similar levels as in Bcrp1-knockout mice; however, the clearance of methotexate in Bcrp1-knockout mice was not reduced by pantoprazole. Further analysis confirmed that pantoprazole reduced methotexate clearance by predominantly inhibiting the hepatobiliary excretion of methotexate by Bcrp1.[68] Thus, inhibition of BCRP may explain the clinical interaction between methotexate and pantoprazole.

Recently, the in vivo pharmacokinetic studies by van Herwaarden et al[62] demonstrated that, at a dose of 1 mg/kg [^{14}C]PhIP, the AUC of oral and IV administration was, respectively, 2.9-fold and 2.2-fold higher in Bcrp1-knockout mice compared with wild-type mice. In mice with cannulated gall bladder, both hepatobiliary and direct intestinal excretion of [^{14}C]PhIP were greatly reduced in Bcrp1-knockout mice compared with the wild-type mice. The data suggest that Bcrp1 effectively restricts the exposure of mice to ingested PhIP by decreasing its absorption from the small intestine and increasing biliary and intestinal elimination. Since PhIP is a food carcinogen that is abundant in the diet, BCRP/bcrp1 is believed to play an important role in protecting from toxicity of normal food constituents.[59,62] The recent study by Polli et al[67] demonstrated that inhibition of Bcrp1 by GF120918 in P-gp-knockout mice increases bioavailability of the NMDA receptor antagonist GV196771. Bcrp1 has also been shown to be directly involved in the renal secretion of organic sulfates in mice.[139] However, BCRP seems unlikely to play a similarly important role in renal secretion of drugs in humans as in mice, since there is little expression of BCRP in human kidney.

Owing to its high level expression in the brain, BCRP is also expected to play an important role in restricting brain penetration of xenobiotics in analogy with P-gp. Van Herwaarden et al[62] measured brain distribution of [^{14}C]PhIP in Bcrp1-knockout mice. In a single time point study, they did not observe a significantly increased brain penetration of PhIP in Bcrp1-knockout mice when corrected for the plasma level, even though Bcrp1 is present in mouse brain vessels. However, a recent study by Cisternino et al[140] demonstrated that Bcrp1 restricted brain uptake of BCRP substrates mitoxantrone and prazosin but not the P-gp substrate vinblastine, suggesting that Bcrp1 at the blood-brain barrier limits brain penetration of its substrates. Further studies are warranted to determine if BCRP indeed functions at the blood-brain barrier, protecting the brain by enhanced efflux of xenobiotics. Taken together, the data shown above suggest that BCRP/Bcrp1 play an important role in drug disposition.

CONCLUSION

Since the discovery of BCRP in 1998, substantial progress has been made in the understanding of drug resistance associated with overexpresssion of this transporter. The substrate specificity of BCRP is overlapping, but clearly distinct from that of P-gp and MRP1. The molecular mechanism by which BCRP acts to transport drugs is still poorly understood at the present time; however, mutational analyses have begun to address the importance of certain residues, particularly the amino acids in the membrane-spanning domain, in determining substrate selectivity by BCRP. Further studies are needed for a clear understanding of the molecular mechanism of this important drug transporter, including determination of a high-resolution 3-dimensional structure that is supported by most of the biochemical evidence. Such studies will provide the molecular basis for developing new ways to circumvent drug resistance in diseases such as cancers. Increasing evidence is now emerging to suggest that BCRP plays an important role in drug disposition. Hence, to predict the effects of BCRP on changes of pharmacokinetics of drugs and the potential drug-drug interactions, it is necessary to determine if the routinely administered drugs such as antibiotics, antiepileptic drugs, antifungal drugs, and antihypertensive drugs are substrates and/or inhibitors of BCRP. The molecular mechanism by which BCRP gene expression is regulated by xenobiotics or special physiological conditions such as pregnancy need also to be explored.

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